

B1
conclude

carbohydrate attached to the conserved site in the C_H2 domain, certain residues in the lower hinge region (eg the sequence ELLGGP (SEQ ID NO:27)) and a proline residue at position 331 and a sequence E-x-K-x-K at positions 318-322. One recent example is disclosed by Cole et al (1997) Journal of Immunology 159, 3613-3621. In that disclosure residues 234, 235 and 237 were mutated to Alanines (or in the case of 235, sometimes to Glu). However these are all unusual residues at these positions in human IgG, thus the presence of such inappropriate amino acids may make the Fc more immunogenic or antigenic and may also lead to the loss of certain desirable Fc functions.

✓
Please replace the paragraph beginning at page 33, line.19, with the following rewritten paragraph:

Figure 17

B2

This shows the Sequences of certain modified and wild-type C_H2 sequences (SEQ ID NO:4-SEQ ID NO:12), including those designated G1Δab (SEQ ID NO:1), G2Δa (SEQ ID NO:2), G1Δac (SEQ ID NO:3).

Please replace the paragraph beginning at page 34,
line 29, with the following rewritten paragraph:

The oligonucleotides used to introduce the mutations were:
between the hinge and CH2 exons

MO10 5' GGA TGC AGG CTA CTC GAG GGC ACC TG 3'. (SEQ ID
NO:13)

between the CH2 and CH3 exons

B3
MO11 5' TGT CCA TGT GGC CCT GGT ACC CCA CGG GT 3'. (SEQ
ID NO:14)

between the CH1 and hinge exons

MO12 5' GAG CCT GCT TCC TCT AGA CAC CCT CCC T 3' (SEQ
ID NO:15)

Restriction sites are underlined.

Please replace the paragraph beginning at page 35,
line 35, with the following rewritten paragraph:

The changes in CH2 at amino acid positions 327, 330 and 331

B4
(Δa mutation) were to be introduced using the
oligonucleotides:-

MO22BACK (coding strand):

5' TCT CCA ACA AAG GCC TCC CGT CCT CCA TCG AGA AAA 3' (SEQ
ID NO:16)

MO22 (complementary strand):

5' TTT TCT CGA TGG AGG ACG GGA GGC CTT TGT TGG AGA 3' (SEQ
ID NO:17)

The changes in CH2 at positions 233 to 236 (Δb and Δc
mutation) were to be introduced using the
oligonucleotides:-

MO7BACK (coding strand and encoding Δc mutation):

5' TCC TCA GCA CCT CCA GTC GCG GGG GGA CCG TCA GTC 3' (SEQ
ID NO:18)

MO21 (complementary strand and encoding Δb mutation):

5' GAC TGA CGG TCC CGC GAC TGG AGG TGC TGA GGA 3' (SEQ ID
NO:19)

The mutations were to be introduced by overlap extension
PCR which also required the oligonucleotides MO11 and
MO10BACK:

5' CAG GTG CCC TCG AGT AGC CTG CAT CC 3' (SEQ ID NO:20)

XhoI restriction site is underlined.

Please replace the paragraph beginning at page 37,
line 30, with the following rewritten paragraph:

The Fog1 variable region DNAs (Bye, J. M., Carter, C., Cui,
Y., Gorick, B. D., Songsivilai, S., Winter, G., Hughes-
Jones, N. C. and Marks, J. D. (1992) Germline variable
region gene segment derivation of human monoclonal anti-

Rh(D) antibodies. J. Clin. Invest. 90, 2481-2490) were obtained in the vector pHEN1. They were amplified by PCR, using the oligonucleotides:-

FOG1VHBACK 5' TCC ACA GGT GTC CAC TCC CAG GTG CAT CTA
CAG CAG 3' (SEQ ID NO:21)

FOG1VHFOR 5' GAG GTT GTA AGG ACT CAC CTG AGG AGA CGG
TGA CCG T 3' (SEQ ID NO:22)

FOG1VKBACK 5' TCC ACA GGT GTC CAC TCC GAC ATC CAG ATG
ACC CAG 3' (SEQ ID NO:23)

FOG1VKFOR 5' GAG GTT GTA AGG ACT CAC GTT TGA TCT CCA
GCT TGG T 3' (SEQ ID NO:24)

25
Cont.
The 5' portion of the insert in the vector M13VHPCR1 (Orlandi, R., Gussow, D. H., Jones, P. T. and Winter, G. (1989) Proc. Natl. Acad. Sci. USA 86, 3833), comprising the promoter and DNA encoding the signal peptide was amplified using the universal M13 reverse primer and VO3:

5' GGA GTG GAC ACC TGT GGA GA 3' (SEQ ID NO:25)

DNA, 3' of the V_H in M13VHPCR1 and representing the 5' end of the V_H-C_H intron, was obtained by PCR using the universal M13 -40 primer and VO4:

5' GTG AGT CCT TAC AAC CTC TC 3' (SEQ ID NO:26)

These two segments of DNA were joined sequentially to both the Fog-1 V_H and Fog-1 V_K amplified DNA by overlap extension PCR as described above. The BamHI restriction site